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Antimicrobial Activity of Cinnamic Acid, Citric Acid, Cinnamaldehyde, and Levulinic Acid Against Foodborne Pathogens

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Introduction

Use of organic acids and essential oils are gaining popularity in the food industry to produce minimally processed food products (Davidson and others 2013). These compounds may be used as natural antimicrobials to inhibit the growth of foodborne pathogens and spoilage microorganisms (Kim and others 1995). Natural antimicrobials may be either added to a food product or already exist in a product to enhance its safety and quality. An ideal natural antimicrobial would inhibit microbial growth at a low concentration while having no effect on the sensory aspects of the food product (Davidson and others 2013).

Animals, plants, and microorganisms all produce natural antimicrobials (Davidson and others 2013). For example, cinnamon is a common culinary herb from which cinnamic acid (3-phenylprop-2-enoic acid) and cinnamaldehyde can be isolated (Deans and Ritchie 1987). Cinnamaldehyde is mainly present in the essential oils of cinnamon which can be found in the bark or leaves (Davidson and others 2013). Citric acid is a 6 carbon tricarboxylic acid and is the primary organic acid in citrus fruits but also can be produced by the filamentous fungus *Aspergillus niger* and the yeast *Yarrowia lipolytica* (Sauer and others 2007). Levulinic acid (4-oxopentanoic acid) is 5 carbon keto acid produced by heating a carbohydrate containing hexose, such as wood, starch, wheat straw, and or cane sugar, with the addition of a dilute mineral acid (Chang and others 2005). Table 1 shows the structure, molecular formula, weight, and solubility of these compounds.

The objective of this experiment was to determine the effects of cinnamic acid, citric acid, cinnamaldehyde, and levulinic acid against *Escherichia coli, Salmonella enterica, and Listeria monocytogenes* by determining the minimal inhibitory concentrations (MIC) in a broth medium.

Materials and Methods

Preparation of inoculum

The bacterial strains *Escherichia coli* O157:H7 BA-1882, *Salmonella enterica* serovar Enteritidis, and *Listeria monocytogenes* ATCC 19111 were obtained from culture collection of the Department of Food Science and Technology at The University of Tennessee, Knoxville. Stock cultures of *E. coli* and *S. enterica* were prepared by inoculating tryptic soy broth (TSB) and incubating for 24 h at 37°C. A loopful of the stock culture was transferred to 10 mL of TSB and incubated at 37°C for 24 h to achieve an approximate 10^9 CFU/ml. Stock cultures of *L. monocytogenes* were prepared by inoculating brain heart infusion broth (BHI) and incubating 32°C for 24 h. A loopful of the stock culture was transferred to 10 mL of BHI and incubated at 32°C for 48 h to achieve an approximate 10^9 CFU/ml. *S. enterica*, *E. coli*, and *L. monocytogenes* were then diluted in broth to 10^4 CFU/ml which was used to inoculate the treatments.

Preparation of antimicrobial agents

Stock solutions (4%) of levulinic (98%; Acros Organics) or citric acid (98%; Fisher Science Education) were prepared in distilled deionized water. The pH of each solution was adjusted to pH 6.0 using NaOH. Stock solutions (10%) of cinnamic acid (98%; Acros Organics) or cinnamaldehyde (98%; SAFC) were prepared in 70% ethanol. All antimicrobial solutions were filter-sterilized using a 0.45 µm membrane filter (Drummond). The solutions were then diluted in sterilied deionized water to 2.0, 1.0, 0.5, 0.25, 0.125, and 0.0625% w/v.

Determination of MIC

The diluted inoculum (4.0 log CFU/ml) and antimicrobial were aseptically transferred into a sterile 96 well microtiter plate (Becton Dickenson). The culture (120 µL) in TSB/BHI was

combined with 120 µL of each antimicrobial concentration for a total of 240 µL per well and a cell concentration of 3.1 log CFU/well. The plate was then incubated at 32°C for 24 h. MIC values were determined by measuring absorbance at 630 nm using a Gen 5 plate reader (BioTek). The final absorbance was subtracted from the initial absorbance to determine the growth. The minimum inhibitory concentration was defined as the lowest concentration of antimicrobial agent that produced an absorbance of < 0.05.

Results and Discussion

The effect of cinnamaldehyde, and cinnamic, citric, and levulinic acids on the growth of *L. monocytogenes* is shown in Table 2 and *E. coli* and *S. enterica* in Table 3. The four antimicrobials showed various degrees of inhibition against the three pathogenic strains. The essential oil component cinnamaldehyde had an MIC against *Listeria monocytogenes* of 0.25%. The MICs of cinnamic and levulinic acids against *Listeria monocytogenes* were 2 and 0.5%, respectively. Citric acid was ineffective at inhibiting *L. monocytogenes* even at 2.0% w/v. Against both *E. coli* and *S. enterica*, cinnamaldehyde had an MIC of 0.25% (Table 3). Similarly to *L. monocytogenes*, cinnamic acid showed an MIC at 2.0% for *E. coli.* In contrast, while there was a decrease in the absorbance compared to the control, cinnamic acid did not completely inhibit the growth of *Salmonella enterica* at 2.0%. Levulinic acid had MICs of 1.0 and 2.0% against *E. coli* and *S. enterica*, respectively. As with *L. monocytogenes*, citric acid had no apparent effect on the growth of either gram-negative pathogen.

Cinnamaldehyde has previously been tested against strains of *E. coli* to determine the minimum inhibitory concentration. The results were similar to that of this experiment where 0.25% cinnamaldehyde was the MIC. (Kim and others 2008). Treatment with 0.25%

cinnamaldehyde caused severe damage to the cell structure inhibiting the growth of the pathogens after 2 h.

In previous studies, citric acid has been shown to be an active antimicrobial but only at lower pHs. For example, the addition of citric acid to tomato juice at pH 4 decreased the aerobic plate count after seven days of storage at 5°C. The control tomato juice had an aerobic plate count of 6.22 log APC/mL while the tomato juice adjusted to a pH 4 using citric acid had an aerobic plate count of 3.80 log APC/mL (Bizri and Wahem, 2006). Since the pH of the citric acid in this study was adjusted to 6.0 the antimicrobial activity was likely negatively affected.

Levulinic acid has previously been tested as an antimicrobial at a pH of 2.67 against *E. coli* O157:H7 and *Salmonella* on meat surfaces. A 2% concentration in a rinse was insufficient at decontaminating surfaces; however, the rinse resulted in a log reduction of 1 log/cm^2 of Salmonella and *E. coli* O157:H7 (Capenter and others 2010). In the present study, we demonstrated that the antimicrobial activity of levulinic acid was retained at a pH of 6.0. This suggests that acidity is not the only cause for the inhibition of foodborne pathogens by levulinic acid.

The results of this experiment further confirm the possibility of using essential oils and organic acids as a natural antimicrobials to control foodborne pathogens such as *E. coli, S. enterica, and L. monocytogenes* and thus potentially reduce foodborne illness. Both levulinic acid and cinnamaldehyde required low concentrations to inhibit the growth of the pathogens.

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Table 1. The structure, molecular formula, solubility, and molecular weight of cinnamaldehyde, cinnamic acid, citric acid, and levulinic acid

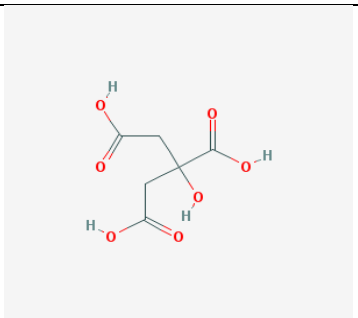
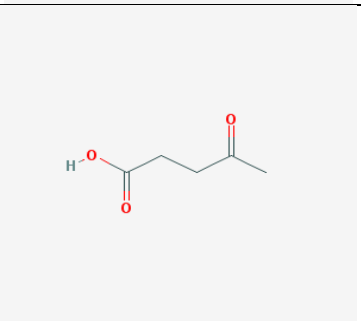
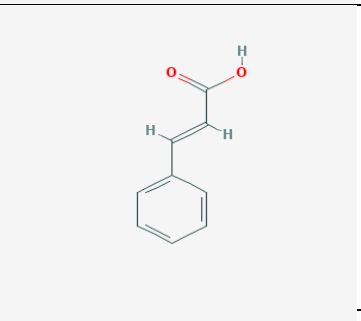
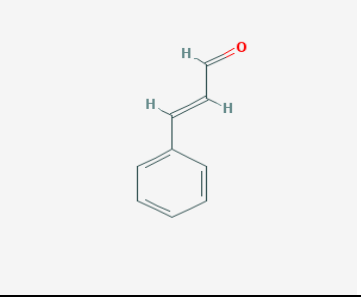
Compound	Structure	Molecular Formula	Molecular Weight (Da)	Solubility (M)
Citric acid		$C_6H_8O_7$	192.12	4.809 M in water
Levulinic acid		$C_5H_8O_3$	116.11	6.746 M in water
Cinnamic acid		$C_9H_8O_2$	148.16	0.004 M in water
Cinnamaldehyde		C_9H_8O	132.16	0.008 M in water

Table 2. Growth of *Listeria monocytogenes* in BHI after 24 h at 32°C in the presence of cinnamaldehyde, cinnamic acid, citric acid and levulinic acid at various concentrations as measured by absorbance at 630 nm. Shaded cells indicated the minimum inhibitory concentration (OD <0.05) of each compound.

Antimicrobial (%)	Cinnamaldehyde	Cinnamic	Citric	Levulinic
Control	0.47±0.03	0.51±0.03	0.86±0.010	0.43±0.05
2.0	0.00±0.00	0.00±0.00	0.43±0.002	0.00±0.00
1.0	0.00±0.00	0.07±0.00	0.61±0.121	0.00±0.00
0.5	0.00±0.00	0.45±0.01	0.62±0.008	0.02±0.02
0.25	0.00±0.00	0.54±0.02	0.57±0.133	0.18±0.01
0.125	0.16±0.00	0.55±0.04	0.61±0.054	0.19±0.01
0.0625	0.41±0.00	0.53±0.01	0.75±0.097	0.23±0.10

Table 3. Growth of *Escherichia coli* or *Salmonella enterica* in TSB after 24 h at 32°C in the presence of cinnamaldehyde, cinnamic acid, citric acid and levulinic acid at various concentrations as measured by absorbance at 630 nm. Shaded cells indicated the minimum inhibitory concentration of each compound.

	Cinnamaldehyde		Cinnamic		Citric		Levulinic	
Antimicrobial (%)	*E. coli*	*S. enterica*	*E. coli*	*S. enterica*	*E. coli*	*S. enterica*	*E. coli*	*S. enterica*
Control	1.07±0.09	1.47±0.02	1.06±0.00	1.47±0.01	0.88± 0.02	0.68±0.02	1.02±0.02	0.96±0.04
2.0	0.08±0.01	0.06±0.00	0.00±0.00	0.39±0.01	0.74±0.12	0.73±0.13	0.00±0.00	0.00±0.00
1.0	0.01±0.00	0.01±0.00	0.35±0.05	0.44±0.00	0.90±0.06	0.90±0.09	0.02±0.01	0.17±0.00
0.5	0.00±0.00	0.00±0.00	0.52±0.01	0.94±0.01	0.97±0.12	0.84±0.01	0.46±0.01	0.53±0.04
0.25	0.00±0.00	0.00±0.01	0.94±0.02	1.30±0.29	0.86±0.07	0.77±0.05	0.60±0.01	0.58±0.10
0.125	0.29±0.02	1.06±0.01	1.10±0.00	1.39±0.05	0.77±0.07	0.72±0.7	0.69±0.13	0.67±0.00
0.0625	0.95±0.11	1.39±.12	1.05±0.01	1.39±0.00	0.76±0.01	0.81±0.03	0.73±0.14	0.71±0.02